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TITLE: Optimal Treatment of Malignant Long Bone Fracture: Influence of Method of Repair and External Beam Irradiation on the Pathway and Efficacy of Fracture Healing

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14. ABSTRACT

The **purpose** of this project is to characterize the differential effects of radiation on the two pathways of fracture healing in an established animal model of bilateral femur fracture repair. Micro CT data obtained from 18 animals in the year 1 SOW (Group I) have been analyzed to assess callus volume and character. Data on RNA isolation and PCR array on 18 animals in the year 1 SOW (Group II) are being analyzed at the partner lab at University of Maryland. Surgical procedures consisting of bilateral femur fracture and repair have been completed on both planned study cohorts (Groups III and IV; total of 40 animals) in the year 2 SOW; all animals were imaged and have been sacrificed according to protocol schedule. Both 6 month (Group III) and 3 month (Group IV) specimens underwent biomechanical testing, and data have been analyzed. Surgical procedures on the bilateral plate group of 9 animals recently added by amendment are being performed. Year 3 SOW animal procedures in Group V for histological testing will begin on schedule in the ninth quarter of the award.

15. SUBJECT TERMS
Fracture healing, bone healing, endochondral ossification, intramembranous ossification, irradiation, radiotherapy, pathologic fractures, bony metastasis, bone cancer, animal model, rat model

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Introduction:

External beam irradiation of malignant lesions metastatic to bone has become a widely accepted therapy to prevent fracture and promote bony healing of lesions that compromise the structural integrity of the skeleton. Likewise, following operative treatment of pathologic fractures, external beam irradiation is utilized to accomplish local tumor kill necessary for disease control, but potentially interferes with the concurrent need to support fracture healing. Such are the competing effects of radiation on bone repair; in *promoting* bone healing while achieving tumor kill in metastatic lesions *before* fracture, while effecting tumor kill *after* pathologic fracture repair radiation may *impair* bone healing. These seemingly competing effects of radiation on osteogenesis and fracture repair are poorly understood. Fractures heal by two different pathways, endochondral or intramembranous ossification, and clinically the pathway followed is determined by the nature of fracture stabilization. Endochondral ossification (EO), "secondary bone healing", is characterized by a cartilaginous callus intermediary induced by interfragmentary motion at the fracture site and occurs with intramedullary devices. Primary bone healing, or intramembranous ossification (IO), results in direct cortical bone repair and requires anatomic reduction and rigid internal fixation with compression plating. There is no consensus on optimal management of pathologic fractures that require external beam irradiation for local tumor kill and little is known about the radiation biology of fracture repair. We propose that there exists an optimal pathway of fracture healing that is relatively less impaired by the effects of radiation therapy, and that this pathway would logically be preferentially exploited in the treatment of pathologic fractures requiring radiation for local tumor control. Further, to the extent that the method of surgical fracture fixation dictates the pathway of fracture healing, we propose that there will likewise exist an optimal method of surgical fracture repair based on the induced biology of fracture healing that would logically be utilized in the setting of malignant skeletal disease where it is anticipated that radiation would be employed as postoperative adjunctive therapy. Accordingly, we will investigate the differential effects of radiation on the two pathways of bone healing and propose an optimal method of surgical fracture repair for managing malignant fractures that require external beam irradiation for local tumor control.

Keywords:
Fracture healing, bone healing, endochondral ossification, intramembranous ossification, irradiation, radiotherapy, pathologic fractures, bony metastasis, bone cancer, animal model, rat model

Overall Project Summary:

Current objectives: **All 72 specimens from 36 study animals in the year 1 SOW (18 in Group I and 18 in Group II) have been processed.** The thirty-six specimens from Group I have been analyzed by micro CT for histomorphometry as well as immunohistochemisty to evaluate callus formation. The thirty-six specimens from Group II have been processed with RNA isolation and PCR array and are currently being

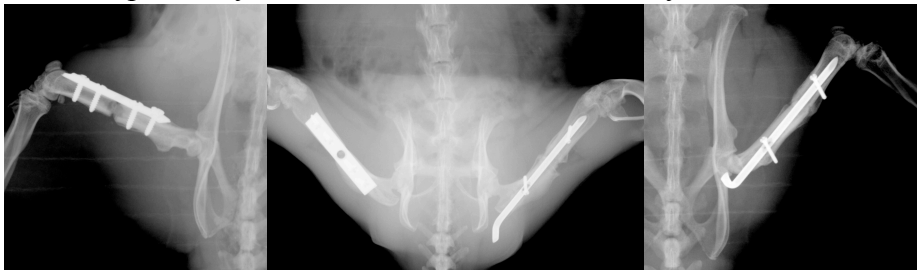

Figure 1. AP and lateral plain film radiographs of Sprague-Dawley rat post bilateral femur fracture procedure.

analyzed to evaluate differential signaling of bone healing. ***Bilateral femur fracture procedures (Figure 1) have been completed on 40 study animals in the year 2 SOW (20 in Group III and 20 in Group IV) as outlined in Quarters 5 and 6 under Task 1.*** All animals have been survived out to predetermined time points, euthanized, and bilateral hind limb tissue harvest has been accomplished producing ***a total of 80 specimens***. All specimens have been analyzed to evaluate biomechanical character. ***A flow chart of all animal groups is included in supplemental appendices, #1***.

Results: The micro CT and histological data obtained from Group I animals have been analyzed. Micro CT images demonstrated a prominent mass of calcified callus (Figure 2a, shown in transparent area) gradually formed from week 1 to week 4 around the fracture sites in femurs repaired by nails. By contrast, femurs repaired by plates demonstrated a thinner layer of calcified callus around the fracture sites (Figure 2a). A significant difference in the volume ratio of calcified callus was observed between the femurs fixed with IM nails and those with plates at week 4 ($p=0.03$, Figure 2b). In addition, a statistically significant decrease ($p=0.042$) in the volume of calcified callus was demonstrated from control (39.76 ± 3.50 mm^3) to the radiation (33.67 ± 8.69 mm^3) groups fixed with IM nails at week 4, representing an approximately 40% decrease in volume of calcified callus in the radiation group (Figure 2c). No statistical difference ($p\geq0.05$) in the volume of calcified callus was observed between the control and radiation groups fixed with plates at any of the studied time points (Figure 2c). In femurs fixed with nails,

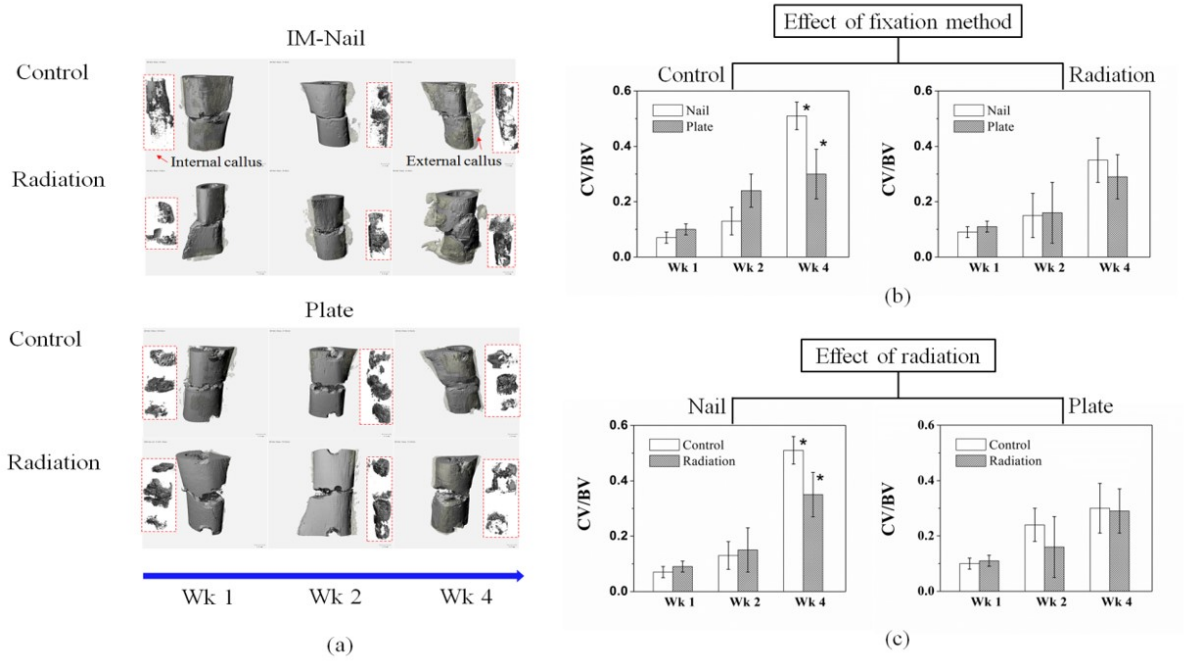

Figure 2. (a) Typical three-dimensional micro-CT images of external and internal callus formed around the fracture sites in rat femurs using nail or rigid plate fixations at week 1, 2 and 4 post-operatively. (b) Effects of fixation methods on the volume ratios of calcified callus in rat femurs of control or radiation groups at week 1, 2 and 4, post-operatively. (c) Effects of radiation on the volume ratios of calcified callus in rat femurs using nail or plate fixations at week 1, 2 and 4, post-operatively.

histological analysis showed that more cartilage tissue was formed around the fractures in control groups (week 2: 37.32 ± 19.88 mm^2; week 4: 39.10 ± 16.28 mm^2) than in the radiation

groups (week 2: 1.54±1.13 mm^2; week 4: 4.60±3.97 mm^2) at both week 2 and 4 ($p<0.0001$; Figures 3a&d). By contrast, no statistical radiation effect ($p\geq0.05$) was found on cartilage formation around the fractures in femurs repaired with plates (Figures 3b&d). The histological images also showed that more cartilage was formed in the femur fixed with nails than that fixed with plates at postoperative week 2 and 4 in control groups (Figure 3a&b). In addition, quantitative tissue histomorphometric analysis at postoperative week 2 and 4 for femurs in control group demonstrated a statistically significant increase in cartilage area around fractures treated by IM nails compared with that fixed with plates ($p<0.0001$; Figure 3c). The micro CT and histological data indicated that fractures treated with nails healed by endochondral ossification and were preferentially impaired by radiation exposure; they exhibited a slower pace of formation and mineralization of cartilage callus and greater ultimate failure of radiographic healing compared to those treated with rigid plates and healed by intramembranous ossification. This work demonstrated that the biological and structural composition of healing fractures differs greatly dependent on the pathway of fracture healing, as determined by the selected method of fracture fixation. Fractures healing via EO demonstrate a more heterogeneous cellular milieu and biological matrix, in the form of cartilage and fibrous tissue, than those healing by an IO pathway. ***Micro CT data acquired from Group I specimens are included in supplemental appendices, #2***.

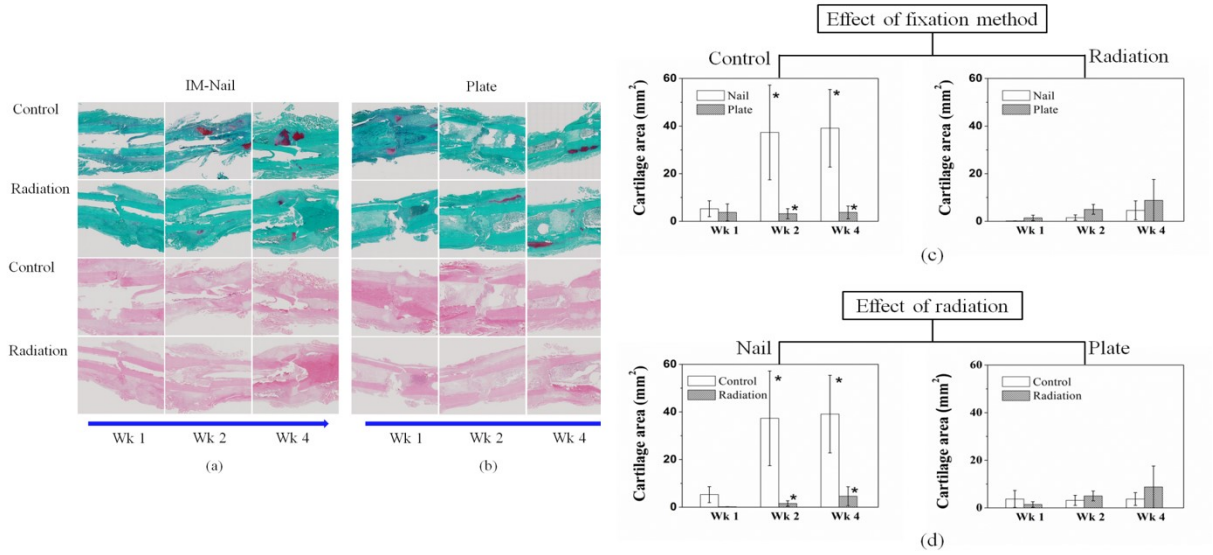

Figure 3. (a-b) Longitudinal cross sections of fractures treated with IM nail or plate fixations at post-operative week 1, 2 and 4. Specimens were stained with Safranin-O & Fast Green and Hematoxylin & Eosin (H&E). The bright red area around the fracture in the figure represented the cartilage while the green area represented fibrous tissue and bone. (c) Effects of fixation methods on the areas of cartilage around fracture sites in rat femurs of control or radiation groups at week 1, 2 and 4, post-operatively. (d) Effects of radiation on the areas of cartilage around fracture sites in rat femurs using nail or plate fixations at various time points post-operatively.

Specimens collected from Group II animals for RNA analysis is currently underway.

Biomechanical testing and analysis have been done on Groups III and IV animals. Two unique breaking patterns were observed with both nailed and plated femurs. Fracture occurred with either a clean snap, accompanied by higher yield force and stiffness, or a partial snap with lower yield force and stiffness (Figure 4). Three dimensional micro CT analysis around fracture

sites further illuminated the intrinsic mechanism of the typical failure patterns (Figure 5): for the nailed femurs with the "clean snap" failure pattern, the fractured bones were fully fused and healed, while with the "partial snap" failure pattern, they were not healed and united with callus connection only, i.e. a fibrous union instead of bony union; for the plated femurs, the fractured bones were fully or partially fused with bridging bone in both instances and fractured with "clean snap" or "partial snap" failure patterns, respectively. Fibrous union could be manually detected in the plated femurs before mechanical testing. In addition, fibrous nonunion was noticed with ten 6-month specimens and seven 3-month specimens. Figure 6 demonstrates the sample counts for the different healing patterns. Several factors may contribute to this, including radiation effect, length of time for fracture healing, surgical technique, and the animal model itself. Therefore, a group of 9 animals has been recently added for biomechanical testing with an approved amendment to undergo bilateral femur fractures with plate fixation on both sides and irradiation on the left while the contralateral limb serves as a non-radiated internal control. The purpose of this modification of study

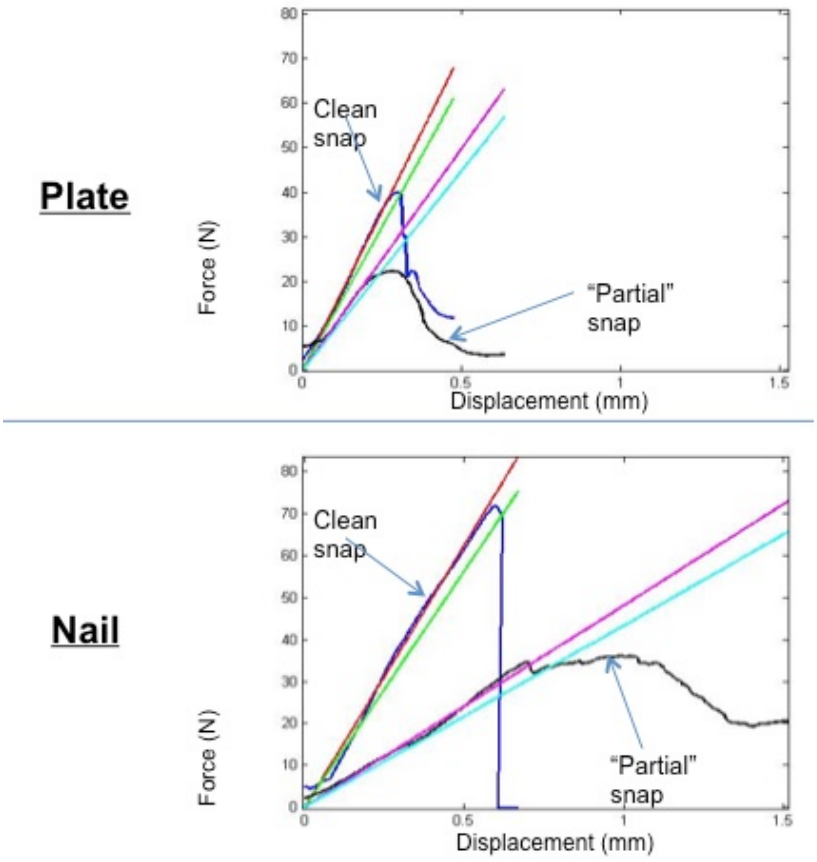

Figure 4. Typical mechanical testing curves of the four types of failure patterns. The slopes of the initial portions of the curves represent stiffness.

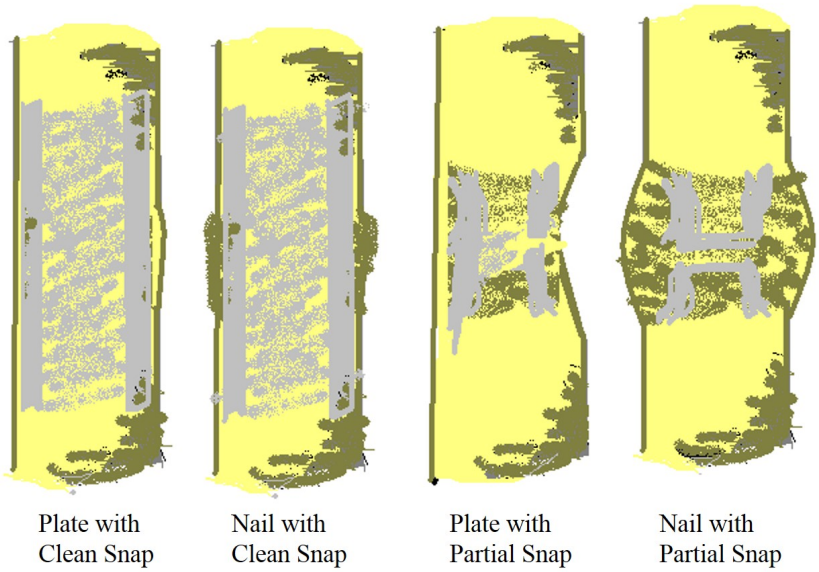

Figure 5. The four types of bone healing patterns observed by micro CT.

protocol is to directly compare results (control vs. irradiated limb) in the same animal in order to avoid confounding variables and further elucidate the true radiation effect on biomechanical strength achieved with the two fracture healing pathways. Furthermore, to eliminate the possibility of excessive motion at the intramedullary nail fracture site as a potential factor for non-bony union, a static interlocking nail with 3 screws was designed to reduce motion and lock the implant more securely than a dynamically compressive nail with 2 screws. Such an amendment to improve the nail implant and change the remaining 6 week mechanical testing group to fixation with bilateral nails and sample harvesting at 12 and 24 weeks has been submitted to, and approved by, both the MUSC IACUC and the DOD ACURO. These protocol modifications will further elucidate the mechanical characteristics of the fractured bones. Once data are obtained on these animals, biomechanical analysis will be combined with the new data to test the preliminary findings. ***Biomechanical testing data acquired from Groups III and IV specimens are included in supplemental appendices, #3***.

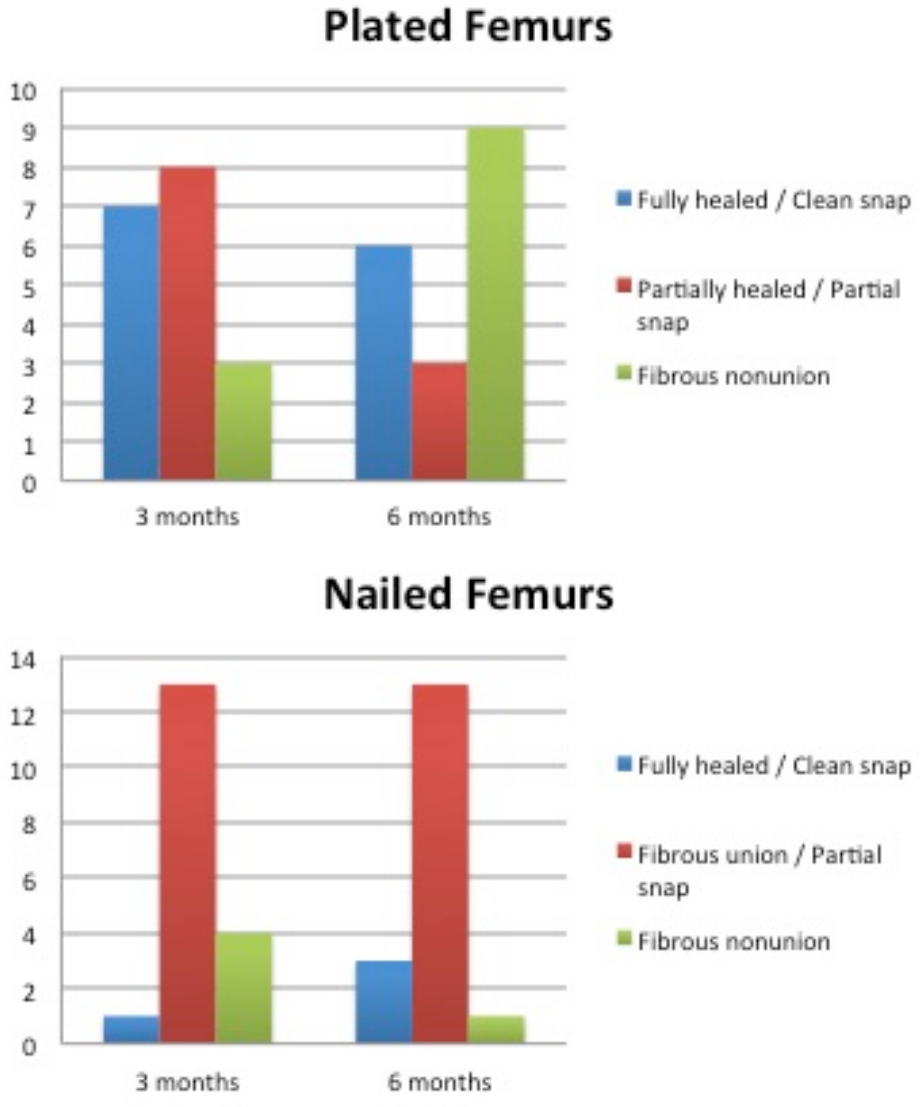

Figure 6. Sample counts on the different healing patterns by plates and nails.

Progress and Accomplishments: The project is on schedule as proposed and planned in the SOW. Bilateral surgical femur fracture procedures on all 76 animals indicated in Task 1 in the SOW for the first two years of the study have been completed. Those harvested specimens have been processed per protocol as delineated under Tasks 2, 3 and 4. Task 1 work specified for the 9th quarter will occur on schedule as planned. We do not anticipate any delays affecting the study in the near future.

Key Research Accomplishments: Animal experiments have been completed on schedule and processing is ongoing with data becoming available on a rolling basis according to tissue processing needs and constraints. Research work specified under Tasks 2, 3 and 4 remain in process. More complete analysis will be performed as data are collected on specimens in year 3 SOW.

Conclusion: Research work is on schedule as proposed and planned. Preliminary research data have prompted protocol modifications to further elucidate hypotheses. Final conclusions and clinical importance will be determined as data analysis is completed in year 3. Nothing to report.

Publications, Abstracts, and Presentations:

Abstracts accepted for Orthopaedic Conferences:

1. Hanna EL, Holmes RE, Wu Y, Robertson A, Barfield WR, Stains JP, Pellegrini VD Jr. *Influence of Method of Repair on the Pathway and Efficacy of Fracture Healing*. The 32nd Annual Meeting of the Southern Orthopaedic Association, Asheville, NC. 2015. (Podium presentation) ***Abstract included in supplemental appendices, #4***.

2. Hanna EL, Holmes RE, Wu Y, Barfield WR, Stains JP, Pellegrini VD Jr. *Influence of the Method of Fracture Repair on the Rate and Completeness of Bone Healing*. The 2015 Annual Meeting of South Carolina Orthopaedic Association, Kiawah Island, SC. 2015. (Podium presentation) ***Abstract included in supplemental appendices, #5***.

3. Wu Y, Hanna EL, McDonald DG, Vanek KN, Yao H, Pellegrini VD Jr. *Effect of External Beam Irradiation on the Pathway of Bone Fracture Healing*. The 2015 Annual Meeting of the Orthopaedic Trauma Association, San Diego, CA. 2015. (Podium presentation) ***Abstract included in supplemental appendices, #6***.

4. Wu Y, Hanna EL, McDonald DG, Vanek KN, Yao H, Pellegrini VD Jr. *Effect of External Beam Irradiation on the Pathway of Bone Fracture Healing*. The 32nd Annual Meeting of the Southern Orthopaedic Association, Asheville, NC. 2015. (2nd place in poster competition) ***Abstract included in supplemental appendices, #7***.

5. Wu Y, Hanna EL, McDonald DG, Vanek KN, Yao H, Pellegrini VD Jr. *Effect of External Beam Irradiation on the Pathway of Bone Fracture Healing*. The 2015 Annual Meeting of South Carolina Orthopaedic Association, Kiawah Island, SC. 2015. (Podium presentation) ***Abstract***

included in supplemental appendices, #8.

Manuscript:

A manuscript entitled, *Effect of External Beam Irradiation on the Pathway of Bone Fracture Healing,* is in preparation and will be submitted to Journal of Bone & Joint Surgery.

Inventions, Patents and Licenses: Nothing to report.

Reportable Outcomes: Nothing to report.

Other Achievements: The experience and training provided by this award during the past year directly contributed to the hiring of the past research resident to a position in the orthopaedic residency at the Medical University of South Carolina. The funding provided by this award contributed to the hiring of the past postdoctoral bioengineering fellow in the Department of Orthopaedics and supported presentations at regional and national Orthopaedic conferences.

References: none

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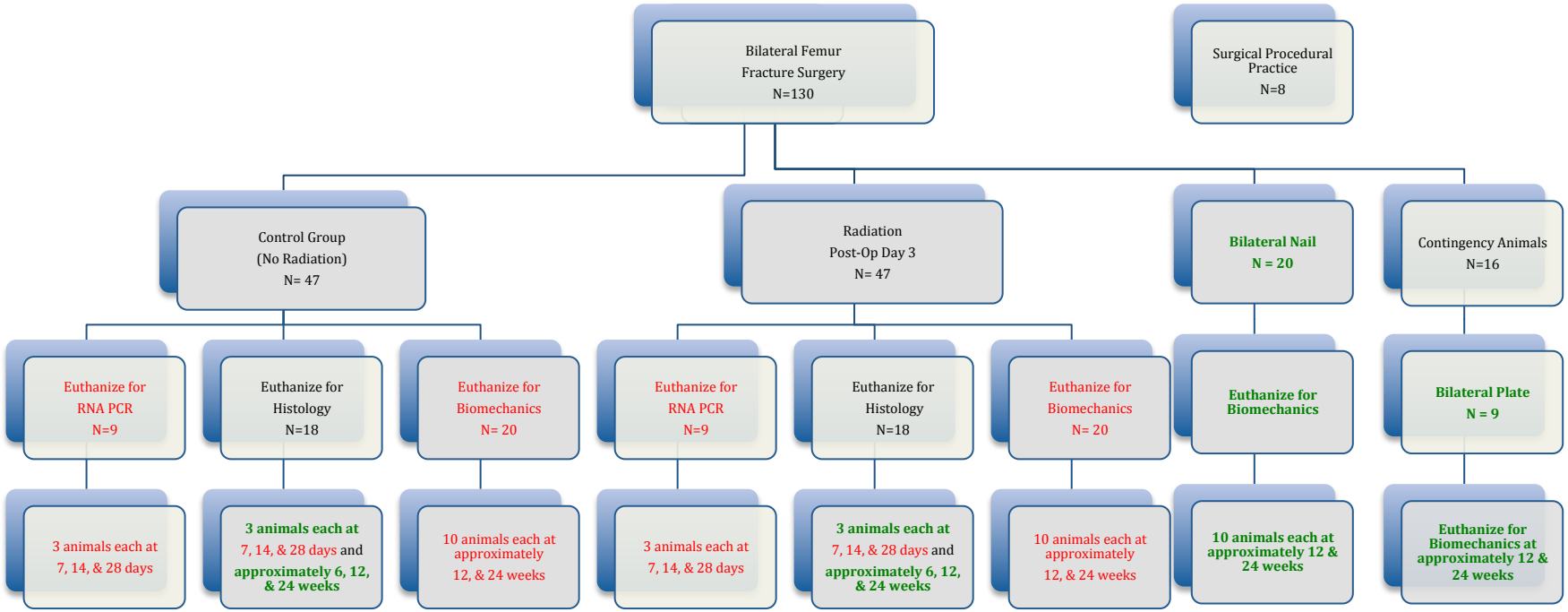
Appendices:

1. Flow Chart of All Animal Groups

a. Year 3 SOW animals, TO BE PERFORMED, are marked in **green**
b. Year 1 & 2 SOW animals, ALL OF WHICH ARE COMPLETED, are marked in **red**



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2. Micro CT Data – Group I animals.

	Sample Information			Callus volume (CV)		Bone Volume (BV)	*True:*		CV /BV		EX/IN	Tissue mineral density (TMD)		Tissue mineral density	D) *True: (mg*	Notes
Co	Plat	1	12	3.64	5.83	79.19	73.	0.	0.0	0.	0.	762.78	1004.	1201.68	1217.33	3D
Co	Plat	1	19	5.70	1.96	82.11	80.	0.	0.0	0.	2.	839.72	880.56	1198.91	1206.69	3D
Co	Plat	1	20	3.72	2.32	71.55	69.	0.	0.0	0.	1.	743.24	846.85	1206.04	1218.08	3D
ntrol	e	wk	**Av**	**4.35**	**3.37**	**77.62**	**74.**	**0.**	**0.0**	**0.**	**1.**	**781.91**	**910.71**	**1202.21**	**1214.03**	reconstruction
			St	*1.17*	*2.14*	*5.45*	*5.51*	*0.*	*0.0*	*0.*	*1.*	*51.01*	*83.15*	*3.59*	*6.37*	
Co	Plat	2	13	17.87	4.30	80.51	76.	0.	0.0	0.	4.	855.74	853.43	1219.00	1239.63	3D
Co	Plat	2	17	10.33	3.04	80.29	77.	0.	0.0	0.	3.	818.52	766.20	1217.70	1235.47	3D
Co	Plat	2	18	13.69	5.21	78.90	73.	0.	0.0	0.	2.	786.48	908.99	1192.61	1212.66	3D
ntrol	e	wk	**Av**	**13.96**	**4.18**	**79.90**	**75.**	**0.**	**0.0**	**0.**	**3.**	**820.25**	**842.87**	**1209.77**	**1229.25**	reconstruction
			St	*3.78*	*1.09*	*0.87*	*1.83*	*0.*	*0.0*	*0.*	*0.*	*34.66*	*71.98*	*14.88*	*14.52*	
Co	Plat	4	6	13.02	3.61	70.05	66.	0.	0.0	0.	3.	882.60	879.54	1069.55	1079.87	3D
Co	Plat	4	7	22.43	8.22	83.59	75.	0.	0.1	0.	2.	894.54	857.78	1125.01	1154.15	3D
ntrol	e	4	16	15.58	3.50	77.99	74.	0.	0.0	0.	4.	881.86	888.06	1208.17	1223.21	reconstruction done.
		wk	**Av**	**17.01**	**5.11**	**77.21**	**72.**	**0.**	**0.0**	**0.**	**3.**	**886.33**	**875.13**	**1134.24**	**1152.41**	
			St	*4.87*	*2.69*	*6.80*	*4.92*	*0.*	*0.0*	*0.*	*0.*	*7.12*	*15.61*	*69.77*	*71.68*	
Co	Nai	1	12	4.70	2.66	89.42	86.	0.	0.0	0.	1.	804.72	838.43	1234.37	1246.51	3D
Co	Nai	1	19	3.72	1.53	91.56	90.	0.	0.0	0.	2.	732.50	863.15	1218.44	1224.48	3D
Co	Nai	1	20	1.99	2.62	88.37	85.	0.	0.0	0.	0.	716.66	942.97	1219.00	1227.43	3D
ntrol	l	wk	**Av**	**3.47**	**2.27**	**89.78**	**87.**	**0.**	**0.0**	**0.**	**1.**	**751.29**	**881.52**	**1223.94**	**1232.81**	reconstruction
			St	*1.37*	*0.64*	*1.63*	*2.24*	*0.*	*0.0*	*0.*	*0.*	*46.94*	*54.64*	*9.04*	*11.96*	
Co	Nai	2	13	13.57	1.67	86.87	85.	0.	0.0	0.	8.	795.46	885.93	1215.85	1222.32	3D
Co	Nai	2	17	6.24	1.12	87.19	86.	0.	0.0	0.	5.	734.63	793.52	1230.20	1235.88	3D
Co	Nai	2	18	9.69	2.09	85.88	83.	0.	0.0	0.	4.	793.43	875.93	1221.96	1230.59	***Nail broken inside.***
ntrol	l	wk	**Av**	**9.83**	**1.63**	**86.65**	**85.**	**0.**	**0.0**	**0.**	**6.**	**774.51**	**851.79**	**1222.67**	**1229.60**	
			St	*3.67*	*0.49*	*0.68*	*1.15*	*0.*	*0.0*	*0.*	*1.*	*34.55*	*50.71*	*7.20*	*6.84*	
Co	Nai	4	6	37.36	1.75	72.14	70.	0.	0.0	0.	21.	850.10	776.11	1154.18	1163.58	3D
Co	Nai	4	7	41.25	2.28	87.16	84.	0.	0.0	0.	18.	820.46	943.43	1139.27	1144.53	3D
Co	Nai	4														3D
ntrol	l	wk	**Av**	**37.78**	**1.98**	**80.75**	**78.**	**0.**	**0.0**	**0.**	**19.**	**831.82**	**877.32**	**1161.16**	**1168.21**	reconstru

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X-	Plat	1	1	7.81	2.77	80.6	77.	0.1	0.	0.	2.82	701.11	740.00	1070.75	1082.51	3D
X-	Plat	1	2	2.81	3.18	71.3	68.	0.0	0.	0.	0.88	725.00	712.50	1078.99	1096.09	3D
X-	Plat	1	8	5.78	2.43	81.5	79.	0.0	0.	0.	2.38	881.76	742.50	1201.96	1216.06	3D
			Av	**5.47**	**2.79**	**77.8**	**75.**	**0.0**	**0.**	**0.**	**2.03**	**769.29**	**731.67**	**1117.23**	**1131.55**	
			St dev	*2.51*	*0.38*	*5.66*	*6.0 1*	*0.0 3*	*0. 01*	*0. 02*	*1.01*	*98.13*	*16.65*	*73.49*	*73.50*	
X-	Plat	2	3	8.34	15.40	99.3	83.	0.1	0.	0.	0.54	781.02	787.22	1065.48	1116.54	3D
X-	Plat	2	9	1.10	3.27	73.4	70.	0.0	0.	0.	0.34	821.95	871.39	1268.72	1287.23	3D
X-	Plat	2	15	8.99	2.91	84.4	81.	0.1	0.	0.	3.09	839.08	715.09	1182.80	1199.50	3D
			Av	**6.14**	**7.19**	**85.7**	**78.**	**0.0**	**0.**	**0.**	**1.32**	**814.02**	**791.23**	**1172.33**	**1201.09**	
			St dev	*4.38*	*7.11*	*12.9 9*	*7.3 3*	*0.0 5*	*0. 08*	*0. 11*	*1.53*	*29.83*	*78.23*	*102.02*	*85.36*	
X-	Plat	4	10	5.15	15.20	98.7	83.	0.0	0.	0.	0.34	940.38	899.36	1123.07	1163.79	3D
X-	Plat	4	11	12.30	1.58	78.7	77.	0.1	0.	0.	7.78	927.41	746.39	1189.93	1199.01	3D
X-	Plat	4	14	14.07	10.90	79.2	68.	0.2	0.	0.	1.29	930.56	851.21	1162.42	1212.05	
			Av	**10.51**	**9.23**	**85.5**	**76.**	**0.1**	**0.**	**0.**	**3.14**	**932.78**	**832.32**	**1158.47**	**1191.62**	
			St dev	*4.72*	*6.96*	*11 .3 6*	*7.6 1*	*0.0 7*	*0. 09*	*0. 09*	*4.05*	*6.76*	*78.21*	*33.60*	*24.96*	
X-	Nai	1	1	4.79	3.55	84.1	80.	0.0	0.	0.	1.35	718.52	778.70	1198.72	1217.22	3D
X-	Nai	1	2	4.44	0.80	81.8	81.	0.0	0.	0.	5.55	779.44	806.02	1144.09	1147.43	3D
X-	Nai	1	8	6.49	2.00	96.4	94.	0.0	0.	0.	3.25	816.39	903.15	1234.00	1241.01	3D
			Av	**5.24**	**2.12**	**87.4**	**85.**	**0.0**	**0.**	**0.**	**3.38**	**771.45**	**829.29**	**1192.27**	**1201.88**	
			St dev	*1.10*	*1.38*	*7.83*	*7.8 4*	*0.0 1*	*0. 02*	*0. 02*	*2.10*	*49.42*	*65.41*	*45.30*	*48.64*	
X-	Nai	2	3	8.66	3.37	89.2	85.	0.1	0.	0.	2.57	819.72	923.15	1156.50	1165.66	***Nail***
X-	Nai	2	9	4.27	0.76	72.2	71.	0.0	0.	0.	5.62	808.98	869.26	1271.22	1275.49	3D
X-	Nai	2	15	15.72	2.68	82.7	80.	0.2	0.	0.	5.87	772.68	699.16	1217.15	1234.49	3D
			Av	**9.55**	**2.27**	**81.4**	**79.**	**0.1**	**0.**	**0.**	**4.68**	**800.46**	**830.52**	**1214.96**	**1225.21**	
			St dev	*5.78*	*1.35*	*8.55*	*7.2 1*	*0.0 7*	*0. 02*	*0. 08*	*1.84*	*24.65*	*11 6.91*	*57.39*	*55.50*	
X-	Nai	4	10	34.07	5.87	68.2	62.	0.5	0.	0.	5.80	884.36	853.34	1132.05	1158.29	3D
X-	Nai	4	11	31.36	1.98	104.	102.	0.3	0.	0.	15.8	841.48	926.30	1204.09	1209.45	3D
X-	Nai	4	14	22.12	0.61	83.6	82.	0.2	0.	0.		851.39	800.19	1196.59	1199.50	3D

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3. Biomechanical Testing Data – Groups III and IV animals.

Sample Information																
Group		Housing Time	Rat Number	Length(mm)	Weight(g)	Stiffness(N/mm)	Yield deflection(mm)	Yield force(N)	Failure Disp(mm)	Maximum Disp(mm)	Maximum load(N)	Post yield deflection(mm)	Plate Gap Size (mm)	Nail Gap Size (mm)	Nail Displacement Size (mm)	Comments
Control	Plate	3 mo	3	40.580	2.036	61.860	0.206	11.481	0.263	0.263	12.980	0.057	0.322			
Control	Plate	3 mo	4	40.310	1.974								0.605			****deleted stiffness for outlier
Control	Plate	3 mo	7	44.300	2.196	69.084	0.652	22.259	0.743	0.653	22.270	0.091	0.399			
Control	Plate	3 mo	8	43.970	2.302	32.118	0.357	10.309	0.440	0.440	11.080	0.084	0.516			
Control	Plate	3 mo	11	43.050	1.944								0.753			Did not heal
Control	Plate	3 mo	12	41.430	2.060	83.2807	0.2224	17.26	0.5584	0.5584	32.83	0.336	0.348			Strange force profile - may have rotated, chose first inflection for yield for
Control	Plate	3 mo	15	42.640	2.375	64.488	0.201	11.679	0.350	0.350	14.410	0.149	0.710			
Control	Plate	3 mo	16	42.460	2.045	33.428	0.194	5.820	0.353	0.353	7.300	0.160	0.397			
Control	Plate	3 mo	19	42.780	2.132	41.388	0.601	20.816	0.606	0.566	22.350	0.005	0.892			
~~Control~~	~~Plate~~	~~3 mo~~	20													moved to histology?
Mean				42.391	2.118	55.092	0.348	14.232	0.473	0.455	17.603	0.126	0.549			
Std Dev				1.384	0.147	19.619	0.199	6.016	0.170	0.142	8.722	0.107	0.202			
Control	Plate	6 mo	1	46.000	2.970								0.494			Did not heal - deleted for outlier euthanized 4/27
Control	Plate	6 mo	2	44.000	2.780								0.863			Did not heal - deleted for outlier euthanized 4/27
Control	Plate	6 mo	5	43.500	2.284	150.750	0.274	37.127	0.311	0.311	39.820	0.037	1.331			
Control	Plate	6 mo	6	46.250	2.588	84.116	0.251	19.019	0.350	0.349	21.490	0.099	1.167			
Control	Plate	6 mo	9	43.330	2.194	53.045	0.483	18.790	0.493	0.477	18.800	0.010	0.541			
Control	Plate	6 mo	10	42.360	2.157								0.551			Did not heal
Control	Plate	6 mo	13	44.170	2.324	102.835	0.499	21.352	0.541	0.541	22.280	0.042	0.406			
~~Control~~	~~Plate~~	~~6 mo~~	14													Moved to histo
Control	Plate	6 mo	17	45.560	2.480	159.862	0.593	35.600	0.593	0.593	35.600	0.000	1.203			
Control	Plate	6 mo	18	44.310	2.149	113.175	1.345	51.350	1.345	1.345	51.350	0.000	1.026			
Mean				44.387	2.436	110.630	0.574	30.540	0.605	0.603	31.557	0.031	0.842			
Std Dev				1.307	0.292	40.298	0.401	13.094	0.378	0.379	12.857	0.038	0.353			
Control	Nail	3 mo	3	39.650	2.560	30.323	1.399	33.060	1.399	1.399	33.060	0.000		0.322	0.312	
Control	Nail	3 mo	4	40.830	2.567									0.605	0.328	Did not heal
Control	Nail	3 mo	7	42.350	2.833	142.779	0.861	70.750	0.898	0.898	71.880	0.038		0.399	1.094	
Control	Nail	3 mo	8	43.430	2.355	60.04	0.7151	33.0485	1.0471	1.0001	36.04	0.332		0.516	1.002	
Control	Nail	3 mo	11	40.260	1.668	25.119	0.7237	15.5238	1.0556	1.0556	19.31	0.3319		0.753	0.546	
Control	Nail	3 mo	12	40.140	1.895	37.994	0.7506	24.6422	1.0637	1.0637	27.33	0.3131		0.348	0.701	
Control	Nail	3 mo	15	42.260	2.370	17.604	1.441	26.500	1.441	1.441	26.500	0.000		0.710	0.814	
Control	Nail	3 mo	16	41.790	2.053									0.397	0.638	Did not heal
Control	Nail	3 mo	19	43.080	1.958	24.197	1.641	28.290	1.641	1.641	28.290	0.000		0.892	0.271	
~~Control~~	~~Nail~~	~~3 mo~~	~~20~~													Moved to histology
Mean				41.532	2.251	48.294	1.076	33.116	1.221	1.214	34.630	0.145		0.549	0.634	
Std Dev				1.363	0.380	43.882	0.401	17.629	0.272	0.277	17.254	0.170		0.202	0.300	
Control	Nail	6 mo	1	47.000	3.350	244.136	0.656	123.700	0.653	0.646	123.830	0.000		0.826	0.676	
Control	Nail	6 mo	2	46.000	3.420	24.343	1.537	27.890	1.537	1.537	27.890	0.000		0.693	0.649	
Control	Nail	6 mo	5	43.270	2.103									0.532	0.598	Not valid - force only 0.8
Control	Nail	6 mo	6	45.650	3.204	18.788	4.132	37.860	3.734	3.734	40.440	0.000		0.248	0.461	
Control	Nail	6 mo	9	43.890	2.792	36.192	1.073	24.890	1.389	1.389	27.740	0.316		0.373	0.925	
Control	Nail	6 mo	10	42.980	2.948	40.559	1.265	39.296	1.476	1.476	44.910	0.211		0.443	0.341	
Control	Nail	6 mo	13	44.200	2.305	27.166	1.407	17.571	1.601	1.848	22.110	0.195		0.641	0.692	
~~Control~~	~~Nail~~	~~6 mo~~	14											0.283	0.807	Moved to histology
Control	Nail	6 mo	17	42.930	2.767	23.814	1.635	30.210	1.685	2.338	31.880	0.051		0.451	0.891	
Control	Nail	6 mo	18	44.030	2.392	196.580	0.904	79.420	0.904	0.904	79.420	0.000				
Mean				44.439	2.809	76.447	1.576	47.605	1.622	1.734	49.778	0.097		0.499	0.671	
Std Dev				1.447	0.470	89.999	1.083	36.008	0.926	0.962	34.895	0.126		0.193	0.191	

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Sample Information															
Group		Housing	Rat Number	Length(mm)	Weight(g)	Stiffness(N/mm)	Yield deflection(mm)	Yield force(N)	Failure Disp(mm)	Maximum Disp(mm)	Maximum load(N)	Post yield deflection(mm)	Plate Gap Size (mm)	Nail Gap Size (mm)	Nail Displacement Size (mm)	Comments
Control	Plate	3 mo	3	40.580	2.036	61.860	0.206	11.481	0.263	0.263	12.980	0.057	0.322			
Control	Plate	3 mo	4	40.310	1.974								0.605			****deleted stiffness for outlier
Control	Plate	3 mo	7	44.300	2.196	69.084	0.652	22.259	0.743	0.653	22.270	0.091	0.399			
Control	Plate	3 mo	8	43.970	2.302	32.118	0.357	10.309	0.440	0.440	11.080	0.084	0.516			
Control	Plate	3 mo	11	43.050	1.944								0.753			Did not heal
Control	Plate	3 mo	12	41.430	2.060	83.2807	0.2224	17.26	0.5584	0.5584	32.83	0.336	0.348			Strange force profile - may have rotated, chose first inflection for yield for
Control	Plate	3 mo	15	42.640	2.375	64.488	0.201	11.679	0.350	0.350	14.410	0.149	0.710			
Control	Plate	3 mo	16	42.460	2.045	33.428	0.194	5.820	0.353	0.353	7.300	0.160	0.397			
Control	Plate	3 mo	19	42.780	2.132	41.388	0.601	20.816	0.606	0.566	22.350	0.005	0.892			
~~Control~~	~~Plate~~	~~3 mo~~	20													moved to histology?
Mean				42.391	2.118	55.092	0.348	14.232	0.473	0.455	17.603	0.126	0.549			
Std Dev				1.384	0.147	19.619	0.199	6.016	0.170	0.142	8.722	0.107	0.202			
Control	Plate	6 mo	1	46.000	2.970								0.494			Did not heal - deleted for outlier euthanized 4/27
Control	Plate	6 mo	2	44.000	2.780								0.863			Did not heal - deleted for outlier euthanized 4/27
Control	Plate	6 mo	5	43.500	2.284	150.750	0.274	37.127	0.311	0.311	39.820	0.037	1.331			
Control	Plate	6 mo	6	46.250	2.588	84.116	0.251	19.019	0.350	0.349	21.490	0.099	1.167			
Control	Plate	6 mo	9	43.330	2.194	53.045	0.483	18.790	0.493	0.477	18.800	0.010	0.541			
Control	Plate	6 mo	10	42.360	2.157								0.551			Did not heal
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Control	Plate	6 mo	17	45.560	2.480	159.862	0.593	35.600	0.593	0.593	35.600	0.000	1.203			
Control	Plate	6 mo	18	44.310	2.149	113.175	1.345	51.350	1.345	1.345	51.350	0.000	1.026			
Mean				44.387	2.436	110.630	0.574	30.540	0.605	0.603	31.557	0.031	0.842			
Std Dev				1.307	0.292	40.298	0.401	13.094	0.378	0.379	12.857	0.038	0.353			
Control	Nail	3 mo	3	39.650	2.560	30.323	1.399	33.060	1.399	1.399	33.060	0.000		0.322	0.312	
Control	Nail	3 mo	4	40.830	2.567									0.605	0.328	Did not heal
Control	Nail	3 mo	7	42.350	2.833	142.779	0.861	70.750	0.898	0.898	71.880	0.038		0.399	1.094	
Control	Nail	3 mo	8	43.430	2.355	60.04	0.7151	33.0485	1.0471	1.0001	36.04	0.332		0.516	1.002	
Control	Nail	3 mo	11	40.260	1.668	25.119	0.7237	15.5238	1.0556	1.0556	19.31	0.3319		0.753	0.546	
Control	Nail	3 mo	12	40.140	1.895	37.994	0.7506	24.6422	1.0637	1.0637	27.33	0.3131		0.348	0.701	
Control	Nail	3 mo	15	42.260	2.370	17.604	1.441	26.500	1.441	1.441	26.500	0.000		0.710	0.814	
Control	Nail	3 mo	16	41.790	2.053									0.397	0.638	Did not heal
Control	Nail	3 mo	19	43.080	1.958	24.197	1.641	28.290	1.641	1.641	28.290	0.000		0.892	0.271	
~~Control~~	~~Nail~~	~~3 mo~~	~~20~~													Moved to histology
Mean				41.532	2.251	48.294	1.076	33.116	1.221	1.214	34.630	0.145		0.549	0.634	
Std Dev				1.363	0.380	43.882	0.401	17.629	0.272	0.277	17.254	0.170		0.202	0.300	
Control	Nail	6 mo	1	47.000	3.350	244.136	0.656	123.700	0.653	0.646	123.830	0.000		0.826	0.676	
Control	Nail	6 mo	2	46.000	3.420	24.343	1.537	27.890	1.537	1.537	27.890	0.000		0.693	0.649	
Control	Nail	6 mo	5	43.270	2.103									0.532	0.598	Not valid - force only 0.8
Control	Nail	6 mo	6	45.650	3.204	18.788	4.132	37.860	3.734	3.734	40.440	0.000		0.248	0.461	
Control	Nail	6 mo	9	43.890	2.792	36.192	1.073	24.890	1.389	1.389	27.740	0.316		0.373	0.925	
Control	Nail	6 mo	10	42.980	2.948	40.559	1.265	39.296	1.476	1.476	44.910	0.211		0.443	0.341	
Control	Nail	6 mo	13	44.200	2.305	27.166	1.407	17.571	1.601	1.848	22.110	0.195		0.641	0.692	
~~Control~~	~~Nail~~	~~6 mo~~	14											0.283	0.807	Moved to histology
Control	Nail	6 mo	17	42.930	2.767	23.814	1.635	30.210	1.685	2.338	31.880	0.051		0.451	0.891	
Control	Nail	6 mo	18	44.030	2.392	196.580	0.904	79.420	0.904	0.904	79.420	0.000				
Mean				44.439	2.809	76.447	1.576	47.605	1.622	1.734	49.778	0.097		0.499	0.671	
Std Dev				1.447	0.470	89.999	1.083	36.008	0.926	0.962	34.895	0.126		0.193	0.191	

4. Abstract #1

Influence of the Method of Fracture Repair on the Rate and Completeness of Bone Healing

[1] Hanna, E; [1] Wu, Y; [1] Holmes, R; [2] Robertson, A; [1] Barfield, W; [1] Pellegrini, V D
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INTRODUCTION

Fractures heal by two different pathways, endochondral (EO) or intramembranous ossification (IO), and clinically the pathway followed is determined by the nature of fracture stabilization. Without a clear understanding of the impact of such factors as smoking, radiation, or the local biology of the host, surgeons more often elect intramedullary nailing of diaphyseal fractures based on technical considerations rather than optimizing the biologic pathway of fracture repair most appropriate for the clinical scenario. Our objective is to characterize the differential features of fracture healing via the EO and IO pathways *in the same animal* by histologic, biomechanical, and radiographic means to assess the comparative rates and completeness of fracture healing.

METHODS

Sprague-Dawley (SD) rats (n=24) have been used to develop a bilateral femur fracture model for concurrent study of both healing pathways of bone in the same animal. One side is repaired with a novel, customized dynamically locked intramedullary device (healing via EO) while the other side is rigidly fixed with plate and screws (healing via IO). At indicated time points the animals were euthanized and their femora harvested for analysis by micro-CT, histology, and biomechanical testing.

RESULTS

The amount of callus formation in femurs repaired using IM nail was consistently greater than observed in those repaired using rigid plate fixation when evaluated with biplanar radiographs.

The volume of calcified callus in the plated group at week 4 was 22.12±7.49 mm^3 compared with 39.76±3.50 mm^3 in the IM group. There is also a significantly greater increase in the callus volume of the IM nail group from week 1 (5.74±2.01 mm^3) to week 4 (39.76±3.50 mm^3) compared to callus volume of the plated group from week 1 (7.72±3.31 mm^3) to week 4 (22.12±7.49 mm^3).

When subjected to biomechanical testing in a four-point bending apparatus, specimens fixed with IM nails exhibited greater early stiffness at 6 weeks (119±28 N/mm IM nail vs. 53±65 N/mm plate) and greater load to failure at 6 (51±16 N/mm IM nail vs. 15±15 N/mm plate) and 12 weeks (77±53 N//mm IM nail vs. 41±18 N/mm plate). However, specimens fixed with plates exhibited greater stiffness (141±110 N/mm IM nail vs. 406±255 N/mm plate) and load to failure (44±47 N/mm IM nail vs. 76±58 N/mm plate) at final 6 month testing.

Tissue histomorphometry at 2 weeks demonstrated a statistically significant increase in overall callus size, cartilage area, and fibrous tissue in fractures treated by IM nailing compared with increased bone area in fractures treated by rigid plate fixation.

DISCUSSION and CONCLUSION

The biological and structural composition of healing fractures differs greatly dependent on the pathway of fracture healing as determined by the selected method of fracture fixation. Through enhanced understanding of the complex differences in these two healing pathways, it may be possible to one day choose the best method of fracture fixation not simply based on the characteristics of the broken bone, but rather with the intent to optimize bony healing by selecting the method of fracture repair that is most appropriate for the biological environment and mitigating factors present in each individual patient

5. Abstract #2

Influence of Method of Repair on the Pathway and Efficacy of Fracture Healing

[1] Hanna, E; [1] Wu, Y; [1] Holmes, R; [1] Barfield, W; [2]Stains, J; [1] Pellegrini, V D
[1] Department of Orthopaedics, Medical University of South Carolina, Charleston, SC, [2] University Of Maryland, Baltimore, MD
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INTRODUCTION
Fractures heal by two different pathways, endochondral (EO) or intramembranous ossification (IO), and clinically the pathway followed is determined by the nature of fracture stabilization. Without a clear understanding of the impact of such factors as smoking, radiation, or the local biology of the host, surgeons more often elect intramedullary nailing of diaphyseal fractures based on technical considerations rather than optimizing the biologic pathway of fracture repair most appropriate for the clinical scenario. Our objective is to characterize the differential features of fracture healing via the EO and IO pathways in the same animal by histologic, biomechanical, and radiographic means to assess the comparative rates and completeness of fracture healing.

METHODS
Sprague-Dawley (SD) rats (n=24) have been used to develop a bilateral femur fracture model for concurrent study of both healing pathways of bone in the same animal. One side is repaired with a novel, customized dynamically locked intramedullary device (healing via EO) while the other side is rigidly fixed with plate and screws (healing via IO). At indicated time points the animals were euthanized and their femora harvested for analysis by micro-CT, histology, and biomechanical testing.

RESULTS
The amount of callus formation in femurs repaired using IM nail was consistently greater than observed in those repaired using rigid plate fixation when evaluated with biplanar radiographs.
The volume of calcified callus in the plated group at week 4 was 22.12±7.49 mm3 compared with 39.76±3.50 mm3 in the IM group. There is also a significantly greater increase in the callus volume of the IM nail group from week 1 (5.74±2.01 mm3) to week 4 (39.76±3.50 mm3) compared to callus volume of the plated group from week 1 (7.72±3.31 mm3) to week 4 (22.12±7.49 mm3).
When subjected to biomechanical testing in a four-point bending apparatus, specimens fixed with IM nails exhibited greater early stiffness at 6 weeks (119±28 N/ mm IM nail vs. 53±65 N/mm plate) and greater load to failure at 6 (51±16 N/mm IM nail vs. 15±15 N/mm plate) and 12 weeks (77±53 N//mm IM nail vs. 41±18 N/mm plate). However, specimens fixed with plates exhibited greater stiffness (141±110 N/mm IM nail vs. 406±255 N/mm plate) and load to failure (44±47 N/ mm IM nail vs. 76±58 N/mm plate) at final 6 month testing.
Tissue histomorphometry at 2 weeks demonstrated a statistically significant increase in overall callus size, cartilage area, and fibrous tissue in fractures treated by IM nailing compared with increased bone area in fractures treated by rigid plate fixation.

DISCUSSION and CONCLUSION
The biological and structural composition of healing fractures differs greatly dependent on the pathway of fracture healing as determined by the selected method of fracture fixation. Through enhanced understanding of the complex differences in these two healing pathways, it may be possible to one day choose the best method of fracture fixation not simply based on the characteristics of the broken bone, but rather with the intent to optimize bony healing by selecting the method of fracture repair that is most appropriate for the biological environment and mitigating factors present in each individual patient.

6. Abstract #3

Effect of External Beam Irradiation on the Pathway of Bone Fracture Healing

[1] Wu, Y; [1] Hanna, E; [2] Mcdonald, D G; [2] Vanek, K N; [3] Yao, H; [1] Pellegrini, V D
+[1] Department of Orthopaedics, Medical University of South Carolina, Charleston, SC,
[2] Department of Radiation Oncology, Medical University of South Carolina, Charleston, SC
[3] Clemson-MUSC Bioengineering Program, Charleston, SC
pellegvd@musc.edu

PURPOSE

External beam irradiation of malignant lesions metastatic to bone has become a widely accepted therapy to prevent fracture and promote bony healing of lesions that compromise the structural integrity of the skeleton. Likewise, following operative treatment of pathologic fractures, external beam irradiation is utilized to accomplish local tumor kill necessary for disease control but potentially interferes with the concurrent need to support fracture healing. We hypothesize that fracture healing by endochondral ossification will be preferentially more impaired than healing by intramembranous ossification in irradiated animals due to radiation-mediated inhibition of the neovascularization and mineralization of cartilage callus during its transition to bone. Therefore, the objective of this study is to investigate the *differential* effects of radiation on the two pathways of bone healing and propose an optimal method of surgical fracture repair for managing malignant fractures that require external beam irradiation for local tumor control.

METHODS

Sprague-Dawley (SD) rats (n=24; male) were used to develop a bilateral iatrogenic femur fracture model for concurrent study of both healing pathways of bone in the same animal. One side was repaired with a novel, customized dynamically locked intramedullary nail (healing via endochondral ossification) while the other side was rigidly fixed with plate and screws (healing via intramembranous ossification) (Figure 1). On postoperative day 3, the rats in the radiation group (n=12) underwent radiation treatment using a customized ¼" thick lead shield with a small aperture to restrict x-ray exposure to only the fracture sites. The PANTAX x-ray unit was operated at 250 kVp, 13 mA with 1.0 mm aluminum plus 0.5 mm copper added filtration (half value layer 1.56 mm copper) to deliver a single dose of 8 Gy to each femur. In order to study the progression of callus formation, the rats in both control and radiation groups were euthanized at various time points (weeks 1, 2 and 4).

The morphology and microstructure of ossification at the fracture site was quantitatively assessed using a Scanco μCT40 system (70kVp, 114mA and 10μm isotropic voxel size). Callus volume (CV), bone volume (BV), callus volume fraction (CV/BV), and tissue mineral density (TMD) were determined with a 3D volumetric reconstruction technique. A Student T-test was used for statistical analysis between control and radiation animals.

RESULTS

A thin layer of calcified callus (Figure 2a, shown in transparent area) gradually formed from week 1 to week 4 around the fracture site in femurs repaired by plate fixation. In the plated femurs, there was no significant difference in the bone volume fraction (CV/BV) of the control group versus radiation group at any of the studied time points (Figure 3a). The volumes of calcified callus in the control and radiation groups at week 4 were 22.12±7.49 mm^3 and 19.73±5.57 mm^3, respectively. By contrast, in femurs repaired by intramedullary (IM) nail fixation a thicker layer of calcified callus formed around the fracture site (Figure 2b). In the IM nail cohort, a significant difference in the bone volume fraction (CV/BV) was observed between control (39.76±3.50 mm^3) and radiation (28.04±7.50 mm^3; $p<0.005$) groups at week 4 (Figure 3b), representing an approximately 40% decrease in bone volume fraction in the radiation group. No statistically significant differences were observed at weeks 1 and 2.

CONCLUSION

This study suggests a differential effect of radiation on the two pathways of bone healing; an insignificant effect on primary bone healing, or intramembranous ossification, as promoted plate fixation, compared with a significant inhibition of endochondral ossification, or secondary bone healing, as occurs with IM nail fixation. A potential explanation for this may be radiation-mediated inhibition of neovascularization and mineralization of cartilage callus during its transition to bone in the endochondral ossification pathway. In conclusion, internal fixation of malignant metastatic fractures with compression plating, rather than intramedullary devices, may be a more appropriate and durable option for fracture repair of pathologic fractures that require external beam irradiation for local tumor control.

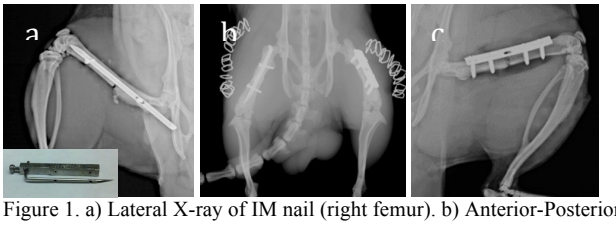


Figure 1. a) Lateral X-ray of IM nail (right femur). b) Anterior-Posterior xray of both femurs. c) Lateral X-ray of plate (left femur).

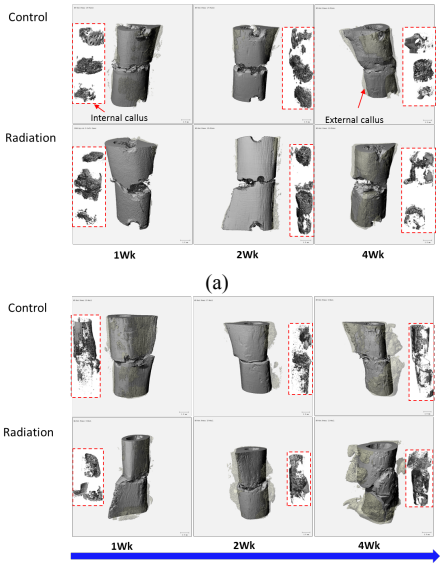
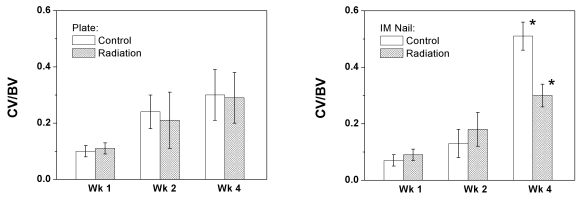


Figure 2. Typical 3D μCT images of external and internal callus formed around the bone fracture site in (a) rat femurs using plate fixation and (b) rat femurs using IM nail fixation at week 1, week 2 and week 4.



(a) (b)

Figure 3. Effects of radiation on the pathways of bone fracture healing in (a) rat femurs using plate fixation and (b) rat femurs using IM nail fixation at various time periods. (*$p<0.005$, n=6/group at week 4)

7. Abstract #4

Effect of External Beam Irradiation on the Pathway of Bone Fracture Healing

[1] Wu, Y; [1] Hanna, E; [2] Mcdonald, D G; [2] Vanek, K N; [3] Yao, H; [1] Pellegrini, V D
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INTRODUCTION

Though external beam irradiation has become a widely accepted method of treating cancerous metastatic lesions that compromise the structural integrity of the skeleton, it's inhibitory effects on the two bone healing pathways remains poorly understood. The objective of this study is to investigate the differential effects of radiation on the two pathways of bone healing and propose an optimal method of surgical fracture repair for managing malignant fractures that require external beam irradiation for local tumor control.

METHODS

Sprague-Dawley (SD) rats (n=24; male) were used to develop a bilateral iatrogenic femur fracture model for concurrent study of both healing pathways of bone in the same animal. One side was repaired with a novel, customized dynamically locked intramedullary nail (healing via endochondral ossification) while the other side was rigidly fixed with plate and screws (healing via intramembranous ossification). On postoperative day 3, the rats in the radiation group (n=12) underwent radiation treatment using PANTAX x-ray unit (250 kVp, 13 mA, 8 Gy). The morphology and microstructure of ossification at the fracture site was quantitatively assessed at weeks 1, 2 and 4.

RESULTS

A thin layer of calcified callus gradually formed from week 1 to week 4 around the fracture site in femurs repaired by plate fixation. In the plated femurs, there was no significant difference in the bone volume fraction of the control group versus radiation group at any of the studied time points. By contrast, in femurs repaired by intramedullary (IM) nail fixation a thicker layer of calcified callus formed around the fracture site. In the IM nail cohort, a significant difference in the bone volume fraction was observed between control and radiation groups at week 4, representing an approximately 40% decrease in bone volume fraction in the radiation group.

DISCUSSION

Our results suggest a differential effect of radiation on the two pathways of bone healing; an insignificant effect on primary bone healing, or intramembranous ossification, as promoted by plate fixation, compared with a significant inhibition of endochondral ossification, or secondary bone healing, as occurs with IM nail fixation. A potential explanation for this may be radiation-mediated inhibition of neovascularization and mineralization of cartilage callus during its transition to bone in the endochondral ossification pathway. Thus, internal fixation of malignant metastatic fractures with compression plating, rather than intramedullary devices, may be a more appropriate and durable option for fracture repair of pathologic fractures that require external beam irradiation for local tumor control.

8. Abstract #5

Effect of External Beam Irradiation on the Pathway of Bone Fracture Healing

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PURPOSE

Though external beam irradiation has become a widely accepted method of treating cancerous metastatic lesions that compromise the structural integrity of the skeleton, its inhibitory effects on the two bone healing pathways remains poorly understood. The objective of this study is to investigate the differential effects of radiation on the two pathways of bone healing and propose an optimal method of surgical fracture repair for managing malignant fractures that require external beam irradiation for local tumor control.

METHODS

Sprague-Dawley (SD) rats (n=24; male) were used to develop a bilateral iatrogenic femur fracture model for concurrent study of both healing pathways of bone in the same animal. One side was repaired with a novel, customized dynamically locked intramedullary nail (healing via endochondral ossification) while the other side was rigidly fixed with plate and screws (healing via intramembranous ossification) (Figure 1). On postoperative day 3, the rats in the radiation group (n=12) underwent radiation treatment using PANTAX x-ray unit (250 kVp, 13 mA, 8 Gy). The morphology and microstructure of ossification at the fracture site was quantitatively assessed at weeks 1, 2 and 4 using a Scanco μCT40 system (70kVp, 114mA and 10μm isotropic voxel size). Callus volume (CV), bone volume (BV), callus volume fraction (CV/BV), and tissue mineral density (TMD) were determined with a 3D volumetric reconstruction technique. To assess the cellular components of healing, the femur tissue was paraffin embedded, coronally sectioned, and mounted on slides. The slides were stained with Safranin O and Fast green for differentiation between cartilage, fibrous tissue, and bone. Histomorphometric quantification of CV/BV and cellular properties such as osteoblast density (ObN/Ar) or osteoclast density (OcN/Ar) were systematically assessed using the Visiopharm software suite.

RESULTS

A thin layer of calcified callus (Figure 2a, shown in transparent area) gradually formed from week 1 to week 4 around the fracture site in femurs repaired by plate fixation. In the plated femurs, there was no significant difference in the bone volume fraction of the control group versus radiation group at any of the studied time points (Figure 3a). By contrast, in femurs repaired by intramedullary (IM) nail fixation a thicker layer of calcified callus formed around the fracture site (Figure 2b). In the IM nail cohort, a significant difference in the bone volume fraction was observed between control and radiation groups at week 4 (Figure 3b), representing an approximately 40% decrease in bone volume fraction in the radiation group.

CONCLUSION

Our results suggest a differential effect of radiation on the two pathways of bone healing; an insignificant effect on primary bone healing, or intramembranous ossification, as promoted by plate fixation, compared with a significant inhibition of endochondral ossification, or secondary bone healing, as occurs with IM nail fixation. A potential explanation for this may be radiation-mediated inhibition of neovascularization and mineralization of cartilage callus during its transition to bone in the endochondral ossification pathway. Thus, internal fixation of malignant metastatic fractures with compression plating, rather than intramedullary devices, may be a more appropriate and durable option for fracture repair of pathologic fractures that require external beam irradiation for local tumor control.

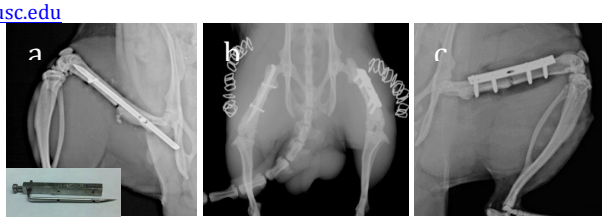


Figure 1. a) Lateral X-ray of IM nail (right femur). b) Anterior-Posterior xray of both femurs. c) Lateral X-ray of plate (left femur).

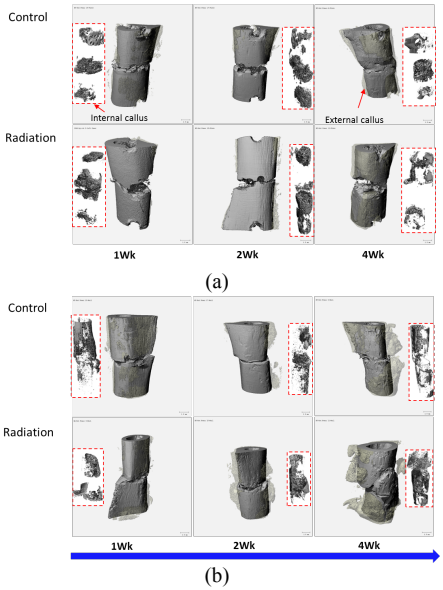
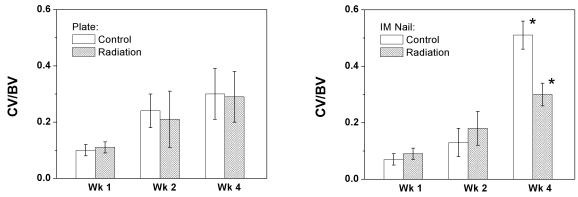


Figure 2. Typical 3D μCT images of external and internal callus formed around the bone fracture site in (a) rat femurs using plate fixation and (b) rat femurs using IM nail fixation at week 1, week 2 and week 4.



(a) (b)

Figure 3. Effects of radiation on the pathways of bone fracture healing in (a) rat femurs using plate fixation and (b) rat femurs using IM nail fixation at various time periods. (n=6/group at week 4)

***ABSTRACT CLASSIFICATION**
k. Tumors/*Metabolic Disease*
l. Basic Research